found, *e.g.*, on page 8, lines 28-30, page 17, line 17 to page 18, line 32 and page 28, line 4 to page 29, line 32 of the specification as filed.

Applicant has amended claim 46 to recite "SEQ ID NO:1" in steps a) and d). Applicant has amended claim 48 to recite "SEQ ID NO:1." The amendments are made to correct an inadvertent typographical error. Support for the amended claims is found, *e.g.*, on page 7, line 24 to page 8, line 2 of the specification as filed.

None of the amendments adds new matter; their entry is requested.

In sum, claims 39-42, 46, and 48-49 are pending.

THE REJECTIONS

Applicant acknowledges with appreciation that the Examiner has withdrawn all other objections and rejections previously stated in Paper No. 8.

35 U.S.C. § 102(e)

Claims 36 and 39-45 stand rejected under 35 U.S.C. § 102(e) for allegedly being anticipated by U.S. Patent 6,171,787 to Wiley ("the '787 patent"). Applicant traverses in part by stating that the Examiner has not met her burden of showing that each element of the pending claims is met by the '787 patent disclosure, by addressing each of the Examiner's allegations in turn, by stating that, as amended, the pending claims are not anticipated by the '787 patent, and by stating that the rejection of claims 36 and 43-45 has been rendered moot by their cancellation herein.

Applicant respectfully states that the Examiner has not met her burden of showing that each element of the pending claims is met by the '787 patent disclosure. *See, Verdegaal Bros. v. Union Oil Co. of California*, 814

5

F.2d 628, 631 (Fed. Cir. 1987) ("A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference."); *see also, generally,* M.P.E.P. § 2131.

The Examiner has provided her view of the scope of the pending claims at pages 2-3 of the instant office action. The Examiner then provided her view of the disclosures of the '787 patent on page 4 of the instant office action that allegedly show how the pending claims are anticipated by the '787 patent. The Examiner, however, has not shown that every element of the pending claims is disclosed in the '787 patent. For example, the Examiner has not shown that the '787 patent teaches every element of amended claims 39 or 40, drawn to a method comprising an anti-APRIL ligand antibody combined with a chemotherapeutic agent or radiation therapy, respectively (*see*, discussion *infra*). Similarly, the Examiner does not show how the '787 patent allegedly anticipates instant claim 42 (*see*, discussion *infra*), drawn to a method comprising exposing a tumor cell that expresses an APRIL receptor to an anti-APRIL ligand antibody. Thus, the Examiner has not met her burden of proving that the '787 patent anticipates the pending claims.

At a minimum, the format of the instant office action makes it difficult to completely understand the basis of the Examiner's rejections. However, applicant shows herein how the Examiner's allegations do not support a finding of anticipation of the pending claims because certain elements, or combinations thereof, recited in the claims, are simply lacking in the Examiner's citations to the '787 patent.

The Examiner alleges that the '787 patent discloses the instant SEQ ID NO:2 as human TNF-gamma ('787 patent SEQ ID NO:2) and soluble human TNF-gamma

('787 patent SEQ ID NO:3). The Examiner also alleges
that the '787 patent discloses antibodies, antagonists
and inhibitors of TNF-gamma that may be used to treat
tumors or metastases, and that these agents can be
combined with radiotherapy or chemotherapeutic agents.
Applicant disagrees.

The '787 patent does not disclose using anti-
TNF-gamma antibodies to treat tumors or metastasis in
combination with radiotherapy or chemotherapeutic agents.
In making this allegation, the Examiner points to column
24, lines 48-52, however, this citation does not support
her contention. Rather, this section of the '787 patent
discloses that "[TNF-gamma] proteins or genes which
encodes their expression may be useful in the prevention
of metastases from the tumors described above either when
used alone or in combination with radiotherapy and/or
chemotherapeutic agents" (col. 24, lines 48-52). Thus,
it is the TNF-gamma protein itself, *and not anti-TNF-
gamma antibodies*, that the '787 patent would combine with
radiotherapy and/or chemotherapy.

As amended, claims 39 and 40 recite a method
that comprises administering to a patient an anti-APRIL
ligand antibody in combination with a chemotherapeutic
agent or radiation therapy. Thus, the '787 patent does
not anticipate claims 39 and 40.

The Examiner alleges that the '787 patent
discloses that "TNF-gamma antibodies, TNF-gamma receptor
antagonists or combinations thereof may be combined with
pharmaceutically acceptable excipeints [*sic*] to form
therapeutic compositions...Wiley discloses the production
of antibodies to TNF-gamma receptor...[and] Wiley
discloses the immunization of animals with the soluble
TNF-gamma of SEQ ID NO:3"

Taken at face value, it is unclear how these
disclosures would anticipate claims 39-42, none of which

7

are composition claims. Claims 39-42 are drawn to
methods of treating cancers and suppressing the growth of
tumor cells. See, *e.g.*, page 18, lines 22-32; page 28,
lines 6-16 and originally filed claims 36-42 on page 41
of the specification as filed. As discussed herein, the
amended claims recite methods that are nowhere taught or
disclosed in the '787 patent. Thus, the disclosures in
the '787 patent cited by the Examiner cannot anticipate
claims 39-42.

The applicant notes that the '787 patent's
generic disclosure of anti-TNF-gamma antibodies cited by
the Examiner does not teach the particular species of
antibodies recited in amended claims 41 and 42. A
species will anticipate a claim to a genus, but only in
certain circumstances will a genus anticipate a species.
See generally, M.P.E.P. § 2131.02. In the chemical arts,
"anticipation can only be found if the classes of
substituents [*i.e.*, species] are sufficiently limited or
well delineated." *Id*. This is not one of those
circumstances as the generic disclosure of anti-TNF gamma
antibodies in the '787 patent is neither sufficiently
limited nor well delineated. Thus, as amended, claims 41
and 42 are not anticipated by the generic anti-TNF-gamma
antibodies disclosed in the '787 patent because claims 41
and 42 are drawn to methods that comprise particular
species of anti-APRIL ligand antibodies.

With respect to claim 42, applicant further
notes that the '787 patent does not teach suppressing the
growth of a tumor cell that expresses a TNF-gamma
receptor with an antibody that binds to TNF-gamma. Thus,
claim 42 cannot be anticipated by the '787 patent.

For at least these reasons, the '787 patent
does not anticipate amended claims 41 and 42.

The Examiner alleges that the '787 patent
"discloses a method of treating conditions associated

with altered or abnormal expression of TNF-gamma
comprising the administration of an antibody which binds
to TNF-gamma...that antagonists can be antibodies...and
that antagonists of TNF-gamma block the inhibitory
effects of TNF-gamma and promote angiogenesis...which
would be conducive to the destruction of metastatic
cancer."

It is unclear which claims the Examiner alleges
are anticipated by these disclosures. Applicant notes
that these disclosures do not contain all of the elements
of any one of the amended claims. For example, these
disclosures do not teach every element of amended claims
39 or 40, drawn to methods comprising an anti-APRIL
ligand antibody combined with a chemotherapeutic agent or
radiation therapy, respectively (see, discussion supra).
The Examiner also does not show how these disclosures
teach every element of amended claim 41, drawn to a
method comprising exposing a tumor cell that expresses an
APRIL ligand polypeptide to an agent consisting of a
particular anti-APRIL ligand antibody species. (see,
discussion supra). Similarly, the Examiner does not show
how these disclosures teach every element of amended
claim 42, drawn to a method comprising exposing a tumor
cell that expresses an APRIL receptor to a particular
anti-APRIL ligand antibody species.

Applicant surmises that the Examiner has
pointed to the '787 patent's disclosure of allegedly
promoting angiogenesis through TNF-gamma antagonism as
reading on at least some of the instant claims. This
disclosure, however, does not teach any of the claimed
methods, and, in fact, teaches away from them.

Many research and development efforts at the
time of filing of the instant application, as well as
today, were focused on *inhibiting* angiogenesis as a means
for controlling cancer, *not promoting angiogenesis*.

9

Angiogenesis is critical "for the growth of solid tumors...thus...antagonists of tumor-associated angiogenesis are needed urgently." *See, Gastl et al., Angiogenesis as a Target for Tumor Treatment, Oncology,* 54:177-184, 182, 1997, attached hereto as Exhibit A. This is because solid tumors require nutrients and oxygen from the bloodstream for their survival. Tumors promote angiogenesis to accomplish this. *Id.* at 177. To treat or restrict a tumor growth, one of skill in the art would know that tumor vascularization would need to be inhibited, which could be achieved by inhibiting angiogenesis. Thus, a person of skill in the art would not read the disclosure relied on by the examiner and believe that the '787 patent teaches any of the claimed methods of treating, suppressing or altering the progression of a cancer, or the claimed methods of suppressing the growth of a tumor cell.

For at least the reasons stated, applicant respectfully requests withdrawal of the rejections of claims 39-42 under 35 U.S.C. § 112. Applicant's cancellation of claims 36 and 43-45 renders the Examiner's rejections of those claims moot.

35 U.S.C. § 112, second paragraph

Claims 46, 48 and 49 stand rejected under 35 U.S.C. § 112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter that applicant regards as the invention. Specifically, the Examiner states that claims 46, 48 and 49 recite a "polypeptide encoded by SEQ ID NO:2," but that SEQ ID NO: 2 is a polypeptide and not a polynucleotide.
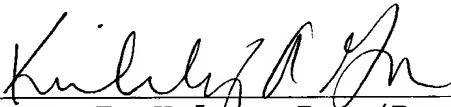
Applicant has amended claims 46 and 48 to recite a "polypeptide encoded by SEQ ID NO:1." The amendment overcomes the Examiner's rejection of claims 46

and 48. The amendment of claim 46 also cures the rejection of claim 49, which depends therefrom. Accordingly, applicant requests that the rejection of 46, 48 and 49 be withdrawn.

CONCLUSION

For the foregoing reasons, applicant believes the claims are in condition for allowance. Applicant requests that the Examiner consider the above remarks, enter the amendments, withdraw the remaining rejections, and allow the pending claims to pass to grant.

Respectfully submitted,

James F. Haley, Jr. (Reg. No. 27,794)
Jonathan M. Kaplan (Reg. No. 46,819)
Attorneys for Applicant
Kimberley A. Gavin (Reg. No. 51,723)
Agent for Applicant
c/o   Fish & Neave
      Customer No. 1473
      1251 Avenue of the Americas
      New York, NY 10020-1104
      Tel.:   (212) 596-9000
      Fax.:   (212) 596-9090
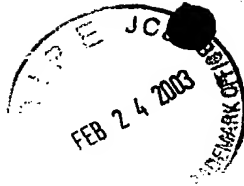
39. (Twice Amended) [The method of Claim 36, wherein said antibody is administered to said patient] <u>A method of treating, suppressing or altering the progression of a cancer, comprising administering to a patient an effective amount of an antibody directed to an APRIL ligand polypeptide capable of interfering with an interaction between said APRIL ligand polypeptide and an APRIL receptor,</u> in combination with a chemotherapeutic agent.

40. (Twice Amended) [The method of claim 36, wherein said antibody is administered to said patient] <u>A method of treating, suppressing or altering the progression of a cancer, comprising administering to a patient an effective amount of an antibody directed to an APRIL ligand polypeptide capable of interfering with an interaction between said APRIL ligand polypeptide and an APRIL receptor,</u> in combination with a radiation therapy.

41. (Twice Amended) A method of suppressing growth of a tumor cell that expresses an APRIL ligand polypeptide, comprising the step of exposing said cell to an effective amount of an agent selected from the group consisting of:

a[.]<u>)</u>   an antibody that binds specifically to said APRIL ligand polypeptide comprising at least about 102 amino acids or a soluble ligand polypeptide thereof;

b[.]<u>)</u>   an antibody that binds specifically to said APRIL ligand polypeptide comprising an amino acid

1

sequence found in amino acids 1-55 of SEQ ID NO:2, or a
fragment thereof; and

c[.]) an antibody that binds specifically
to said APRIL ligand polypeptide comprising an amino acid
sequence found in amino acids 157-250 of SEQ ID NO:2, a
fragment thereof, or a soluble ligand polypeptide
thereof.[;

d. an antibody that binds specifically to SEQ
ID NO:2 or a soluble ligand polypeptide thereof; and

e. an antibody that binds specifically to
said APRIL ligand polypeptide and blocks an interaction
between said APRIL ligand polypeptide and an APRIL
receptor polypeptide.]

42. (Twice Amended) A method of suppressing
growth of a tumor cell that expresses an APRIL receptor
polypeptide, comprising the step of exposing said cell to
an effective amount of an agent selected from the group
consisting of:
a[.]) an antibody that binds specifically
to an APRIL ligand polypeptide comprising at least about
102 amino acids or a soluble ligand polypeptide thereof;

b[.]) an antibody that binds specifically
to an APRIL ligand polypeptide comprising an amino acid
sequence found in amino acids 1-55 of SEQ ID NO:2, or a
fragment thereof; and

c[.]) an antibody that binds specifically
to an APRIL ligand polypeptide comprising an amino acid
sequence found in amino acids 157-250 of SEQ ID NO:2, a

2

fragment thereof, or a soluble ligand polypeptide
thereof.[;

d. an antibody that binds specifically to SEQ
ID NO:2 or a soluble ligand polypeptide thereof; and

e. an antibody that binds specifically to an
APRIL ligand polypeptide and blocks an interaction
between said APRIL ligand polypeptide and said APRIL
receptor polypeptide.]

46. (Amended) A method for identifying an agent
capable of suppressing the growth of a cell culture,
comprising the steps of:

a) identifying a cell that proliferates
in response to the binding of a polypeptide encoded by
SEQ ID [NO:2] NO:1 to its cell surface receptor;

b) growing said cell in said cell
culture;

c) exposing said cell culture comprising
said cell to a compound resulting in an exposed cell
culture;

d) concurrently or subsequently exposing
said exposed cell culture to [a] said polypeptide encoded
by SEQ ID [NO:2] NO:1, or a fragment thereof;

e) comparing the proliferation of said
cell within said exposed cell culture to the
proliferation of a substantially identical cell in a
second cell culture that was not exposed to said
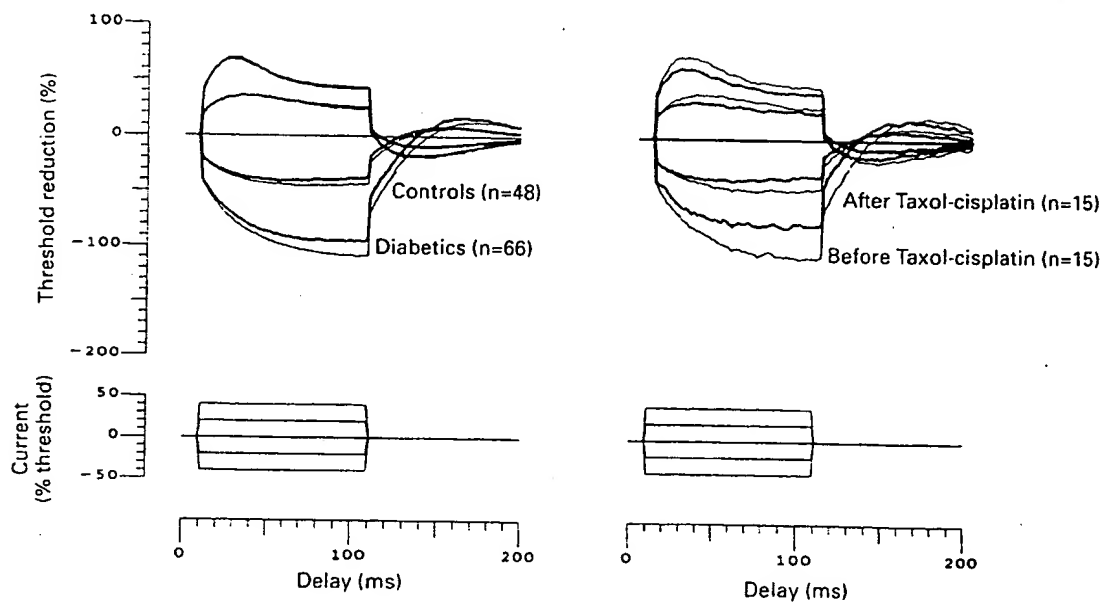compound; and

f)   determining whether said compound has suppressed the growth of said cell in said exposed cell culture.

48.   (Amended) The method of claim 46, wherein said compound is an antibody directed to the polypeptide encoded by SEQ ID [NO:2] NO:1, or a fragment thereof.

International Journal of Cancer Research and Treatment

Threshold reduction (%)

Controls (n=48)

Diabetics (n=66)

After Taxol-cisplatin (n=15)

Before Taxol-cisplatin (n=15)

Current (% threshold)

Delay (ms)

Delay (ms)

EXPRESS MAIL
EV133111608US

Review

Günther Gastl[a]
Thomas Hermann[b]
Michael Steurer[b]
Jörg Zmija[b]
Eberhard Gunsilius[a]
Clemens Unger[b]
Andrea Kraft[b]

[a] Division of Hematology and
Hemato-Oncology, Department of
Internal Medicine, University of Innsbruck,
Austria; and
[b] Department of Medical Oncology,
Tumor Biology Center, Freiburg, Germany

Angiogenesis as a Target for Tumor Treatment

Abstract
Angiogenesis is a key step in tumor growth, invasion and metastasis. Thus, antiangiogenic therapy was postulated to be an attractive approach for antitumor treatment. Based on today's knowledge, at least three strategies for inhibition of angiogenesis are feasible: (1) inhibition of release of angiogenic factors from tumor cells and/or neutralization of angiogenic molecules that have already been released; (2) inhibition of vascular endothelial cell proliferation and migration, and (3) inhibition of the synthesis and turnover of vessel basement membrane. To date, a number of antiangiogenic agents have been identified. In animal models, treatment with angiogenesis inhibitors has proven antitumor effects. Early clinical experience with angiogenic inhibitors indicates that optimal antiangiogenic therapy in the future is likely to be based on the long-term administration to cancer patients in adjunct to surgery, radiotherapy and conventional chemotherapy.

Inhibition of angiogenesis was first postulated as an anticancer strategy by Judah Folkman [1] in 1971. Since then the discovery of angiogenic factors, their receptors and multiple negative regulators of angiogenesis has revolutionized our understanding of neovascularization. Thus, the pivotal role of angiogenesis in the behavior of human tumors is becoming well established. This review summarizes the recent progress that has been made in the development of antiangiogenic therapy in oncology.

The Role of Angiogenesis in Neoplastic Disease

The progressive growth of solid tumors is strictly dependent on their ability to stimulate formation of new blood vessels that will supply tumor cells with oxygen and essential nutrients. Under normal physiological conditions, the formation of new blood vessels is tightly repressed. The molecular mechanisms by which a solid tumor induces its own blood supply are increasingly better understood [2–4]. One consequence of the cascade of

Günther Gastl, MD
Division of Hematology and Hemato-Oncology
Department of Internal Medicine, University Hospital
Anichstrasse 34
A–6020 Innsbruck (Austria)

Table 1. Endogenous mediators regulating angiogenesis [reviewed in 7]

Angiogenic stimulators	Angiogenic inhibitors
Fibroblast growth factors	Angiostatin
Vascular endothelial growth factor	Interferons
Angiogenin	Platelet factor 4
Transforming growth factor-α	Thrombospondin
Tumor necrosis factor-α	Prolactin
Platelet-derived endothelial cell growth factor	bFGF soluble receptor
Transforming growth factor-β[1]	Transforming growth factor-β[1]
Placental growth factor	Tissue inhibitors of metalloproteinases (TIMPs)
Interleukin-8	Placental proliferin-related peptide
Hepatocyte growth factor	Glioma-derived angiogenesis inhibitory factor
Platelet-derived growth factor	
Granulocyte colony-stimulating factor	
Proliferin	
Prostaglandins (PGE$_1$, PGE$_2$)	

[1] Although TGF-β has been shown to inhibit growth of most endothelial cells in vitro, it is clearly angiogenic in vivo, possibly directly by increasing thrombospondin, an extracellular matrix-associated antiangiogenic protein, or directly via chemoattraction and activation of other cells.

oncogene activation and tumor suppressor gene loss that produces a solid tumor is the alleviation of this normal suppression [5]. Most tumors in humans persist for months to years without neovascularization until a subset of tumors cells acquires an angiogenic phenotype. The switch to the angiogenic phenotype involves a change in the local equilibrium between positive and negative regulators of angiogenesis [6] (table 1). Tumor cells may overexpress angiogenic factors, may mobilize angiogenic proteins from the extracellular matrix, may recruit host cells such as macrophages which then produce their own angiogenic molecules, or may engage in a combination of these processes [7].

Up-regulation of angiogenic factors, however, is not sufficient in itself for a tumor cell to become angiogenic. Certain negative regulators of angiogenesis may need to be down-regulated [6–9]. Such endogenous inhibitors normally defend the vascular epithelium from mitogenic stimuli. The formation of new blood vessels is not only a prerequisite for local tumor growth and expansion but also significantly influences the metastatic spread of malignant cells. It has been shown that greater numbers of blood vessels at the tumor site increase the opportunity for malignant cells to enter the circulation [10]. Moreover, newly formed capillaries have fragmented basement membranes and are leaky, making them more penetrable by tumor cells than mature vessels. Several clinical studies have shown that the degree of tumor angiogenesis is relat-

ed to clinical outcome, suggesting that angiogenic properties correlate with tumor aggressiveness and metastatic potential [reviewed in 11].

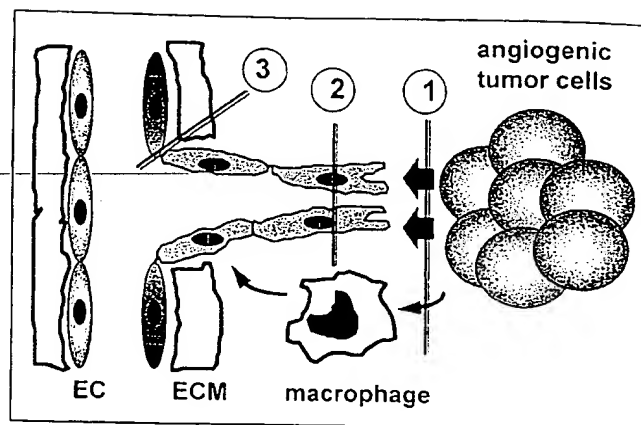## Rationale and Approaches for Antiangiogenic Tumor Therapy

The principal target for antiangiogenic therapy is represented by proliferating endothelial cells. In normal tissues endothelial cells are quiescent, whereas in tumors they are activated and proliferating. Based on the current knowledge of tumor neovascularization the following antiangiogenic strategies are currently under investigation: (1) neutralization of angiogenic peptides and/or stimulation or administration of natural angiogenesis inhibitors; (2) inhibition of endothelial cell proliferation, migration and formation of new blood vessels, and (3) inhibition of the synthesis and turnover of vessel basement membrane (fig. 1, table 2).

In this respect it is important to distinguish between antiangiogenesis and vascular targeting. The former interferes with a wide range of biological processes involved in growth, migration and differentiation of blood vessels. The latter provides rapid destruction and cell death of the vessels, infarcting large areas of the tumor. For clinical application, vascular targeting will require the identification of target molecules that are present at sufficient den-

Gastl/Hermann/Steurer/Zmija/Gunsilius/
Unger/Kraft

Table 2. Classification of angiogenic inhibitors

Agent	Reference
Inhibitors and endogenous antagonists of angiogenic growth factors	
Antibodies to bFGF	Hori et al. [12]
Antibodies to VEGF	Kim et al. [13]
Angiogenin antagonist	Olson et al. [14]
Suramin and analogs	Braddock et al. [15]
	Takano et al. [16]
	Myers et al. [17]
Sulfonic derivatives of distamycin A	Tanaka et al. [18]
Recombinant platelet factor 4	Maione et al. [19]
Angiostatin	O'Reilly et al. [20]
Retinoids	Majewski et al. [21]
Vitamin D and analogs	Oikawa et al. [22]
	Shokravi et al. [23]
Inhibitors of endothelial cell growth and/or migration	
AGM-1470	Antoine et al. [24]
(angioinhibin or TNP-470)	Ingber et al. [25]
Quinoline-3-carboxamide (linomide)	Vukanovic et al. [26]
Tamoxifen	Gagliardi and Collins [27]
D-Penicillamine	Matsubara et al. [28]
Genistein	Fotsis et al. [29]
Thalidomide	D'Amato et al. [30]
Interferons	Sidky and Borden [31]
Interleukin-12	Voest et al. [32]
Angiogenic inhibitors targeting the basement membrane and extracellular matrix	
Antibody anti-αvβ3	Brooks et al. [33]
Metalloproteinase inhibitors	Davies et al. [34]
(e.g. batimastat)	
Minocycline	Tamargo et al. [35]
PAI-1	Mueller et al. [36]
Aurintricaboxylic acid	Gagliardi and Collins [37]
Potential new antiangiogenic therapeutics	
Nitric oxide synthase inhibitor	Ziche et al. [38]
Thrombospondin antagonist	Weinstat-Saslow et al. [39]
fps/fes inhibitor	Greer et al. [40]

Fig. 1. Current strategies of antiangiogenic therapy include (1) blockage of angiogenic factors (arrows), (2) inhibition of endothelial cell (EC) growth and migration, and (3) suppression of the synthesis and degradation of vessel basement membrane and extracellular matrix.

and flt-1 [44] and VEGF itself [45]. Vascular targeting has been recently reviewed [46, 47]. This review focuses on antiangiogenic therapy.

Inhibitors of Angiogenic Peptides

Angiogenic peptides can be blocked by neutralizing antibodies, soluble receptor molecules or various synthetic compounds (table 2). Of the factors that stimulate angiogenesis, the heparin-binding growth factors (HBGFs) are the best characterized and include basic fibroblast growth factor (bFGF) and VEGF. Monoclonal antibodies or antagonistic molecules neutralizing bFGF [12], VEGF [13] or angiogenin [14] showed significant antitumor activity in animal models. A number of highly sulfated compounds that bind HBGFs such as suramin and its analogs [14–16] and sulfonic derivatives of distamycin A [18] are currently in early clinical trials. Suramin is a polysulfonated naphthylurea originally developed as treatment of African trypanosomiasis. In laboratory experiments, suramin was able to prevent receptor binding of a number of growth factors including the angiogenic peptides bFGF and platelet-derived growth factor (PDGF). The drug has been tested in hormone-refractory prostate cancer [17] and objective responses have been reported. Takano et al. [16] recently demonstrated that suramin is able to inhibit multiple control points of angiogenesis: binding of bFGF to its endothelial receptor, endothelial cell migration and proliferation and the activ-

sity on the surface of vascular endothelial cells in solid tumors but absent from endothelial cells in normal tissues. Such target molecules could be used to develop antibodies to target cytotoxic agents to the vascular endothelial cells of the tumor rather than to the tumor cells themselves. Some promising candidate target molecules have already been identified in humans, including endoglin [41], endosialin [42], a fibronectin isoform [43], the vascular endothelial growth factor (VEGF) receptors KDF

ity of urokinase-type plasminogen activator. However, with the doses required to achieve antitumor activity in humans, severe systemic toxicity was observed. To overcome this problem, structurally related polyanions are presently under preclinical evaluation at the Imperial Cancer Research Laboratories of the University of Oxford [15]. Angiostatic steroids such as 11α-hydrocortisone and 17α-hydroxyprogesterone lack gluco- and mineralocorticoid activity and may potentiate the activity of anti-HBGF agents through mechanisms unrelated to inhibition of inflammation-induced angiogenesis [48]. So far, these steroids have not been tested in humans but conventional glucocorticoids cause regression of hemangiomas if injected directly into the lesion or if given at high doses systemically [7].

Platelet factor 4 (PF4) is a 7,800-kD platelet α-granule protein. PF4 has been reported to inhibit angiogenesis and growth factor-stimulated endothelial cell proliferation [19]. In vitro, human recombinant PF4 seems to inhibit the mitogenic activity of VEGF on endothelial cells by various mechanisms [49]. Intralesional administration of PF4 resulted in regression of injected lesions in patients with Kaposi's sarcoma [50]. Angiostatin, a 38-kD fragment of plasminogen is a specific inhibitor of endothelial growth and a potent angiogenesis inhibitor, which, when administered systemically to mice, can hold metastases in a dormant state [9]. Angiostatin also suppresses the growth of primary tumors more potently than other angiogenic inhibitors. In SCID mice treated with angiostatin, human tumor xenografts can be held dormant at a microscopic size [20].

Differentiation-inducing agents such as retinoids and vitamin D analogs inhibited tumor cell-induced angiogenesis in vitro and in vivo [21–23]. Simultaneous administration of retinoids and 1.25-dihydroxyvitamin D3 led to a synergistic inhibition of tumor-associated angiogenesis in mice [51]. Very recently, these compounds have been shown to induce [52, 53] and act in concert with natural angiogenic inhibitors such as interferons [54, 55].

Inhibitors of Endothelial Cell Growth and Migration

Several drugs have been developed that specifically inhibit endothelial cell proliferation and/or migration (table 2). Fumagillin is a naturally secreted antibiotic from the fungus *Aspergillus fumigatus*. Fumagillin has been shown to have antiproliferative effects against endothelial cells in culture and to inhibit angiogenesis in the chick embryo-chorionallantoic membrane assay [24] but is toxic to animals. TNP-470, a synthetic analogue of fumagillin, was found to be less toxic and a more potent inhibitor of angiogenesis [25]. TNP-470 is a potent inhibitor of endothelial cell proliferation and migration, suppresses capillary tube formation, and has antiproliferative activity against human glioblastoma cells in vivo [56]. In xenotransplanted human colorectal cancer, a significant antimetastatic effect was demonstrated on the hepatic metastases of both orthotopical and subcutaneous tumors [57].

Phase I studies with this drug have been approved by the Food and Drug Administration and are currently in progress. Preliminary pharmacokinetic data suggested a rapid clearance of TNP-470 from the blood with a plasma half-life of only 5–10 min. As with most antiangiogenic agents, more prolonged exposure to inhibitory drug concentration may be required for maximal efficacy. Other synthetic compounds such as quinoline-3-carboxamide [26], tamoxifen [27], D-penicillamine [28], genistein [29] or thalidomide [30] have also shown biological activity in angiogenesis assays. Interferons have been reported to inhibit the growth of endothelial cells in vitro to block lymphocyte-induced angiogenesis in vivo [31] and to suppress the production of bFGF in human tumor cells [58]. Therapy with interferon-α was found to effectively induce regression of life-threatening hemangiomas in infants, which often overexpress bFGF [59]. One of the newest members of antiangiogenic compounds is interleukin-12. Interleukin-12 inhibited almost completely corneal neovascularization in mice. This potent suppression of angiogenesis was dependent on the induction of IFN-γ [32].

Inhibitors of Extracellular Matrix Formation and Turnover

One of the most profound influences of the angiogenic process is the endothelial cell interaction with the vascular basement membrane and the extracellular matrix. The composition of the extracellular matrix contributes to the morphology, proliferation and differentiation of endothelial cells [60]. Thus, another target of antiangiogenic treatment is the vessel basement membrane. The inhibition of its synthesis and turnover can effectively block the formation of neovessels. Several drugs act at this level (table 2). Extracellular matrix contains some proteins, such as laminins and integrins, that are required for neovascularization. Recently, Brooks et al. [33] have shown that the vascular integrin $\alpha_v\beta_3$ plays a crucial role in angiogenesis.

Gastl/Hermann/Steurer/Zmija/Gunsilius/
Unger/Kraft

These authors demonstrated that it is possible to induce apoptosis of proliferative angiogenic blood vessels by a specific antibody to the $\alpha_v\beta_3$ integrin.

Neovascularization also depends on the degradation of vessel basement membrane and local degradation of extracellular matrix proteins. Matrix metalloproteinases (MMPs) (subclassified as collagenases), stromelysins or gelatinases can degrade basement membrane glycoproteins and all of the components of extracellular matrix [61]. These enzymes, which are closely associated with angiogenesis, are up-regulated in proliferating endothelial cells. MMPs exist in latent proenzyme and active enzyme forms and require zinc for their activity. Batimastat (BB-94) is a low molecular peptide mimetic that reversibly binds to zinc in the active site of MMPs [34]. In animal models, batimastat inhibited tumor growth, invasion and metastasis in well-tolerated doses. Clinical trials with this drug are in progress. Minocycline and other tetracycline derivatives with anticollagenase activity have also been shown to be potent inhibitors of angiogenesis [35]. Other protease inhibitors with antiangiogenic activity such as plasminogen activator 2 (PAI-2) [36] and aurintricarboxylic acid [37] have been recently reported.

Other Potential Targets for Inhibition of Angiogenesis

Based on recent experiments to understand the pro- and antiangiogenic pathways, a series of potential new targets are available for antiangiogenic therapy. These targets include procoagulant pathways [39], tyrosine kinases [40] involved in endothelial growth, and nitric oxide synthase [37] (table 2).

Selection and Monitoring of Tumor Patients Undergoing Antiangiogenic Therapy

Currently, assessment of vascular density in tumor tissue is a research tool but may become important in the future to select patients for antiangiogenesis therapy. However, the evaluation of microvessel density in primary tumor specimens cannot be used for in vivo monitoring of therapeutic effects or give an overall assessment of angiogenesis in metastases. Thus, it will be necessary to develop methods to monitor antiangiogenic agents in patients. In vivo methods may be based on biochemical markers of angiogenesis such as the determination of angiogenic peptides in blood, urine or malignant effusions

[62, 63]. Monitoring may be further based on blood flow measurement in tumor lesions, e.g. by color Doppler sonography [64] or uptake of radioisotopes [65], and by positron emission tomography scanning [66]. Radioisotope-labeled monoclonal antibodies specific for proliferating endothelial cells or tumor-blood-vessels [41–43] may be another useful method for scanning of primary and metastatic tumor lesions.

Notes from in vivo Studies for Future Antiangiogenic Therapy

From animal studies and clinical trials of antiangiogenic therapy, the following preliminary conclusions can be drawn and tested as hypotheses in future trials:

(1) Antiangiogenic therapy is mainly directed at migrating and proliferating cells in capillaries at sites of neovascularization. Thus, specific inhibitors of angiogenesis should be well tolerated in most tumor patients because under physiologic conditions angiogenesis is limited to wound healing and reproduction.

(2) Inhibitors of angiogenesis generally down-regulate neovascularization by inhibiting the proliferation and locomotion of endothelial cells rather than by killing the cells. In the design of clinical trials it may therefore be necessary to administer antiangiogenic drugs for a prolonged period of time, perhaps for several months to years.

(3) Since inhibitors of angiogenesis act through diverse mechanisms of action, antiangiogenic agents may achieve maximum biologic activity when administered together [67].

(4) To date, resistance to antiangiogenic compounds has not been a problem in long-term studies.

(5) As antiangiogenic therapy may only halt tumor growth but not produce regressions, disease stabilization may be the maximum that can be achieved in trials of advanced cancer. Hence, ways to monitor inhibition of tumor growth, reduction in vascularity, decreased production of angiogenic factors or increased production of endogenous angiogenic inhibitors may be surrogate marker endpoints.

(6) Potential strategies to enhance the activity of angiogenic inhibitors are to use them in combination with cytotoxic drugs or radiotherapy [68, 69]. In animals with tumors, combinations of antiangiogenic and cytotoxic compounds can be curative, whereas the effect of either agent alone is merely inhibitory [68]. The use of antiangiogenic drugs together with chemotherapy may further

be important to inhibit regrowth of tumors between courses of therapy. Radiotherapy is also potentiated by antiangiogenic therapy in tumor-bearing animals, partly because antiangiogenic therapy decreases hypoxia in the tumor [67].

(7) Angiogenic inhibitors could also be used after conventional tumor therapy, when patients enter a remission. They might then keep micrometastases in a dormant state. Such dormancy has already been tested in animals that have continued to have excellent health despite the presence of dormant micrometastases [9, 20].

(8) One frequent argument against antiangiogenesis as a potential means to treat solid tumors is that of the heterogeneity of tumor vascularity. It is conceivable, however, that even avascular tumor areas may still be amenable to antiangiogenic therapies. First, by blocking neovascularization growth of a small avascular tumor nodule is rather limited, metastasis becomes unlikely and such metastatic foci as do develop may remain quiescent. Secondly, additional targeting of an already-formed vasculature in the marginal zone of an otherwise hypovascular tumor lesion may indirectly cause necrosis of the central tumor area by reducing the diffusion of oxygen and essential nutrients and limiting catabolites to exit.

Conclusions

Angiogenesis is critical not only for the growth of solid tumors but also for cell shedding from the primary tumor and the metastatic spread to distant sites. Thus, the identification, development and clinical testing of antagonists of tumor-associated angiogenesis are needed urgently. This need is underscored by convincing evidence from recent reports that the degree of microvessel density in primary tumor lesions correlates with time to metastasis and survival in patients. Recently, a number of antiangiogenic agents have been developed as pharmaceuticals and are currently being tested in clinical studies. Early clinical trials have appropriately proceeded in a manner similar to that used for traditional cytotoxic agents to define dose-limiting toxicities and to determine a schedule of drug administration for further efficacy testing. However, unlike cytotoxic chemotherapy, antiangiogenic agents may need to be administered continuously at well-tolerated dose levels that do not require interruption of drug administration. Clinical monitoring of biologic endpoints may prove the key in the rational development of antiangiogenic compounds. With little theoretical risk of inducing second malignancies, these agents are particularly attractive for use as novel adjuvants to conventional treatment of primary malignancies for patients who are at high risk for local tumor recurrence and metastatic disease.

References

1 Folkman J: Tumor angiogenesis: Therapeutic implications. N Engl J Med 1971;285:1182–1186.

2 Folkman J: Tumor angiogenesis: in Mendelsohn J, Howley PM, Israel MA, Liotta LA (eds): The Molecular Basis of Cancer. Philadelphia, Saunders, 1995, pp 206–232.

3 Bicknell R, Harris AL: Mechanisms and therapeutic implications of angiogenesis. Curr Opin Oncol 1996;8:60–65.

4 Marmè D: Tumor angiogenesis. The pivotal role of vascular endothelial growth factor. World J Urol 1996, in press.

5 Bouck N: Angiogenesis: a mechanism by which oncogenes and tumor suppressor genes regulate tumorigenesis. Cancer Treat Res 1992;63:359–371.

6 Dameron KM, Volpert OV, Taisnky MA, Bouk N: Control of angiogenesis in fibroblasts by p53 regulation of thrombospondin-1. Science 1994; 265:1582–1584.

7 Folkman J: Clinical applications of research on angiogenesis. N Engl J Med 1995;333:1757–1763.

8 Van Meir EG, Poverini PJ, Chazin VR, Su Huang HJ, de Tribolet N, Cavenee WK: Release of an inhibitor of angiogenesis upon induction of wild-type p53 expression in glioblastoma cells. Nat Genet 1994;8:171–176.

9 Holmgren L, O'Reilly MS, Folkman J: Dormancy of micrometastases: Balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nat Med 1995:1:27–31.

10 Fidler IJ, Ellis LM: The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell 1994;79:185–188.

11 Gasparini G, Harris AL: Clinical importance of the determination of tumor angiogenesis in breast carcinoma: Much more than a new prognostic tool. J Clin Oncol 1995;13:765–782.

12 Hori A, Sasada R, Matsutani E, Naito K, Sakura Y, Fujita T, Kozei Y: Suppression of solid tumor growth by immunoneutralizing monoclonal antibody against human basic fibroblast growth factor. Cancer Res 1991;51:6180–6184.

13 Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N: Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 1993;362:841–844.

14 Olson KA, Fett JW, French TC, Key ME, Vallee BL: Angiogenin antagonists prevent tumor growth in vivo. Proc Natl Acad Sci USA 1995; 92:442–446.

15 Braddock PS, Hu De, Fan TDP, Stratford IJ, Harris AL, Bicknell R: A structure-activity analysis of antagonism of the growth factor and angiogenic activity of basic fibroblast growth factor by suramin and related polyanions. Br J Cancer 1994;69:890–898.

16 Takano S, Gately S, Neville ME, Herblin WF, Gross JL, Engelhard H, Perricone M, Eidsvoog K, Brem S: Suramin, an anticancer and angiosuppressive agent, inhibits endothelial cell binding of basic fibroblast growth factor, proliferation, and induction of urokinase-type plasminogen activator. Cancer Res 1994;54:2654–2660.

Gastl/Hermann/Steurer/Zmija/Gunsilius/ Unger/Kraft

17 Myers C, Cooper M, Stein C, LaRocco R, Walther MM, Weiss G, Choyke P, Dawson N, Steinberg S, Uhrich MM, Cassidy J, Kohler DR, Trepel J, Linehan MW: Suramin: a novel growth factor antagonist with activity in hormone-refractory prostate cancer. J Clin Oncol 1992;10:881–889.

18 Tanaka NG, Sakamoto N, Inoue K, Korenaga H, Kadoya S, Ogawa H, Osada Y: Antitumor effect of an antiangiogenic polysaccharide from an *Arthrobacter* species with or without a steroid. Cancer Res 1989;49:6727–6730.

19 Maione TE, Gray GS, Petro J, Hunt AJ, Donner AL, Bauer SI, Carson HF, Sharpe RJ: Inhibition of angiogenesis by recombinant human platelet factor 4 and related peptides. Science 1990;247:77–79.

20 O'Reilly MS, Holmgren L, Chen C, Folkman J: Angiostatin induces and sustains dormancy of human primary tumors in mice. Nat Med 1996;2:689–692.

21 Majewski S, Polakowski I, Marczak M, Jablonska S: The effect of retinoids on lymphocyte- and transformed cell line-induced angiogenesis. Clin Exp Dermatol 1986;2:317–318.

22 Oikawa T, Hirotani K, Ogasawara H, Katayama T, Nakamura O, Iwaguchi T, Hiragun A: Inhibition of angiogenesis by vitamin D₃ analogues. Eur J Pharmacol 1990;178:247–250.

23 Shokravi MT, Marcus DM, Alroy J, Egan K, Saomil MA, Albert DM: Vitamin D inhibits angiogensis in transgenic murine retinoblastoma. Invest Ophthalmol Vis Sci 1995;36:83–87.

24 Antoine N, Greimers R, De-Roanne C, Kusaka M, Heinen E, Simar LJ, Castronovo V: AGM-1470, a potent angiogenesis inhibitor, prevents the entry of normal but not transformed endothelial cells into the G1 phase of the cell cycle. Cancer Res 1994;54:2073–2076.

25 Ingber D, Fujita T, Kishimoto S, Sudo K, Kanamaru T, Brem H, Folkman J: Angioinhibins: synthetic analogues of fumagillin which inhibit angiogenesis and suppress tumour growth. Nature 1990;348:555–557.

26 Vukanovic J, Passaniti A, Hirata T, Traystman RJ, Harley-Asp B, Isaacs JT: Antiangiogenic effects of the quinoline-3-carboxamide linomide. Cancer Res 1993;53:1833–1837.

27 Gagliardi A, Collins DC: Inhibition of angiogenesis by antiestrogens. Cancer Res 1993;53:533–535.

28 Matsubara T, Saura R, Hirohata K, Ziff M: Inhibition of human endothelial cell proliferation in vitro and neovascularization in vivo by D-penicillamine. J Clin Invest 1989;83:158–167.

29 Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R, Schweigerer L: Genistein, a dietary-derived inhibitor of in vitro angiogenesis. Proc Natl Acad Sci USA 1993;90:2690–2694.

30 D'Amato RJ, Loughman MS, Flynn E, Folkman J: Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci USA 1994;91:4082–4085.

31 Sidky YA, Borden EC: Inhibition of angiogenesis by interferons: Effects on tumor- and lymphocyte-induced vascular responses. Cancer Res 1987;47:5155–5161.

32 Voest EE, Kenyon BM, O'Reilly MS, Truitt G, D'Amato RJ, Folkman J: Inhibition of angiogenesis in vivo by interleukin-12. J Natl Cancer Inst 1995;87:581–586.

33 Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA: Integrin αvβ3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell 1994;79:1157–1164.

34 Davies B, Brown PD, East N, Crimmin MJ, Balkwill FR: A synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice with human ovarian cancer xenografts. Cancer Res 1993;53:2087–2091.

35 Tamargo RJ, Bok RA, Brem H: Angiogenesis inhibition by minocycline. Cancer Res 1991;51:672–675.

36 Mueller BM, Yu YB, Laug WE: Overexpression of plasminogen activator inhibitor 2 in human melanoma cells inhibits spontaneous metastasis in scid/scid mice. Proc Natl Acad Sci USA 1995;92:205–209.

37 Gagliardi AR, Collins DC: Inhibition of angiogenesis by aurintricarboxylic acid. Anticancer Res 1994;14:475–479.

38 Ziche M, Morbidelli L, Masoni E, Amerini S, Granger HJ, Maggi CA, Gepetti P, Ledda F: Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. J Clin Invest 1994;94:2036–2044.

39 Weinstat-Saslow DL, Zabrenetzky VS, Van Houtte K, Frazier WA, Roberts DD, Steeg PS: Transfection of thrombospondin complementary DNA into a human breast carcinoma cell line reduces primary tumor growth, metastatic potential and angiogenesis. Cancer Res 1994;54:6504–6511.

40 Greer P, Haigh J, Mbamalu G, Khoo W, Bernstein A, Pawson T: The fps/fes protein tyrosine kinase promotes angiogenesis in transgenic mice. Mol Cell Biol 1994;14:6755–6763.

41 Burrows FJ, Derbyshire EJ, Tazzari PL, Amlot P, Gazdzar AF, King SW, Letarte M, Vitetta ES, Thorpe PE: Up-regulation of endoglin on vascular endothelial cells in human solid tumors: Implications for diagnosis and therapy. Clin Cancer Res 1995;1:1623–1634.

42 Rettig WJ, Garinchesa P, Healey JH, Su SL, Jaffe EA, Old LJ: Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer. Proc Natl Acad Sci USA 1992;89:10832–10836.

43 Carnemolla B, Balza E, Siri A, Zardi L, Nicrotra MR, Bigotti A, Natali PG: A tumor-associated fibronectin isoform generated by alternative splicing of messenger RNA precursors. J Cell Biol 1989;108:1139–1148.

44 Plate KH, Breier G, Weich HA, Risau W: Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 1992;359:845–848.

45 Dvorak HJ, Sioussat TM, Brown LF, Berse B, Nagy JA, Sotrel A, Manseau EJ: Distribution of vascular permeability factor (vascular endothelial growth factor) in tumors – Concentration in tumor blood vessels. J Exp Med 1991;174:1275–1278.

46 Bicknell R: Vascular targeting and the inhibition of angiogenesis. Ann Oncol 1994;5:45–50.

47 Fan TP, Jaggar R, Bicknell R: Controlling the vasculature: Angiogenesis, anti-angiogenesis and vascular targeting. Trends Pharmacol Sci 1995;16:57–66.

48 Folkman J, Ingber DE: Angiostatic steroids: Method of discovery and mechanism of action. Ann Surg 1987;206:373–384.

49 Gengrinovitch S, Greenberg SM, Cohen T, Gitay-Goren H, Rockwell P, Maione TE, Levi BZ, Neufeld G: Platelet factor-4 inhibits the mitogenic activity of VEGF₁₂₁ and VEGF₁₆₅ using concurrent mechanisms. J Biol Chem 1995;270:15059–15065.

50 Staddon A, Henry D, Bonnem E: A randomized dose finding study of recombinant human platelet factor-4 in cutaneous AIDS-related Kaposi's sarcoma (abstract). Proc Am Soc Clin Oncol 1994;13:50.

51 Majewski S, Szmurlo A, Marczak M, Jablonska S, Bollag W: Inhibition of tumor cell-induced angiogenesis by retinoids, 1,25-dihydroxyvitamin D₃ and their combination. Cancer Lett 1993;75:35–39.

52 Pienta KJ, Nguyen NM, Lehr JE: Treatment of prostate cancer in the rat with the synthetic retinoid fenretinimide. Cancer Res 1993;53:224–226.

53 Lingen MW, Polverini PJ, Bouck NP: Synergy between retinoic acid and interferon-α in inhibiting angiogenesis induced by head and neck squamous cell carcinoma (abstract). Proc Am Assoc Cancer Res 1996;37:60.

54 Moore DM, Kalvakolanu DV, Lippman SM, Kavanagh JJ, Hong WK, Borden EC, Paredes-Espinoza M, Krakoff IH: Retinoic acid and interferon in human cancer: Mechanistic and clinical studies. Semin Hematol 1994;31:31–37.

55 Bollag W: Experimental basis of cancer combination chemotherapy with retinoids, cytokines, 1,25-dihydroxyvitamin D₃, and analogs. J Cell Biochem 1994;56:427–435.

56 Takamiya Y, Brem H, Ojeifo J, Mineta T, Martuza RL: AGM-1470 inhibits the growth of human glioblastoma cells in vitro and in vivo. Neurosurgery 1994;34:869–875.

57 Konno H, Tanaka T, Kanai T, Maruyama K, Nakamura S, Baba S: Efficacy of an angiogenesis inhibitor, TNP-470, in xenotransplanted human colorectal cancer with high metastatic potential. Cancer 1996;77:1736–1740.

58 Singh RK, Gutman M, Bucana CD, Sanchez R, Llansa N, Fidler IJ: Interferons α and β downregulate the expression of basic fibroblast growth factor in human carcinomas. Proc Natl Acad Sci USA 1995;92:4562–4566.

59 Ezekowitz RAB, Mulliken JB, Folkman J: Interferon-alpha-2a therapy for life-threatening hemangiomas of infancy. N Engl J Med 1992;326:1456–1463.

60 Lawrence JA, Steeg PS: Mechanisms of tumor invasion and metastasis. World J Urol 1996, in press.

61 Matrisan LM: Metalloproteinases and their inhibitors in matrix remodelling. Trends Genet 1990;6:121–125.

62 Nguyen M, Watanabe H, Budson AE, Richie JP, Hayes DF, Folkman J: Elevated levels of an angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. J Natl Cancer Inst 1994;86: 356–361.

63 Kraft A, Weindel K, Marth C, Schumacher P, Zmija J, Hermann T, Steurer M, Unger C, Marmè D, Gastl G: Vascular endothelial growth factor in serum and malignant ascites (abstract). Proc Am Assoc Cancer Res 1996;37: 58.

64 Wu CC, Lee CN, Chen TM, Shyn MK, Hsieh CY, Chen HY, Hsieh FJ: Incremental angiogenesis assessed by color Doppler ultrasound in the tumorigenesis of ovarian neoplasms. Cancer 1994;73:1251–1256.

65 Scopinaro F, Schillaci O, Scarpini M, Mingazzini PL, Di Macio L, Banci M, Danieli R, Zerili M, Limiti MR, Cent-Colella A: Technetium sestamibi: An indicator of breast cancer invasiveness. Eur J Nucl Med 1994;21:984–987.

66 Abramovitch R, Meir G, Neeman M: Neovascularization induced growth of implanted C6 glioma multicellular spheroids: Magnetic resonance microimaging. cancer Res 1995;55: 1956–1962.

67 Teicher BA, Holden SA, Ara G, Sotomayor EA, Huang ZD, Chen YN, Brem H: Potentiation of cytotoxic cancer therapies by tnp-470 alone and with other antiangiogenic agents. Int J Cancer 1994;57:920–925.

68 Kato T, Sato K, Kakinuma H, Matsuda Y: Enhanced suppression of tumor growth by combination of angiogenesis inhibitor o-(chloroacetylcarbamoyl)fumagillol (TNP-470) and cytotoxic agents in mice. Cancer Res 1994;54: 5143–5147.

69 Teicher BA, Holden SA, Gulshan A, Korbut T, Menon K: Comparison of several antiangiogenic regimens alone and with cytotoxic therapies in the Lewis lung carcinoma. Cancer Chemother Pharmacol 1996;38:169–177.

Gastl/Hermann/Steurer/Zmija/Gunsilius/Unger/Kraft